From Responsive Chemistry to History-Bearing Molecular Systems

A condition, record, reader, and return architecture for the emergence of history-bearing molecular systems

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Theory and mechanistic synthesis · 26 September 2026

Abstract. Primordial chemistry was shaped by environmental variation before molecular systems could regulate their own reaction conditions. Prebiotic experiments now connect several steps relevant to a transition toward history-bearing chemistry. Relevant building blocks can arise under overlapping chemistries; RNA can be assembled and copied in environmentally structured regimes; and small RNAs can acquire catalytic capacity. Polymerization then introduces path dependence because each product changes the chemical context of the next reaction.

The framework developed here asks when such responsive chemistry becomes history-bearing. A condition record exists when a molecular consequence of an earlier condition persists after that condition ends and still distinguishes which prior condition occurred under common later conditions. A reader makes the retained difference consequential under common later conditions, and a return occurs when that consequence changes a variable governing another write. The evidence is therefore organized by causal role rather than by a presumed historical sequence.

The manuscript organizes prebiotic, synthetic and extant mechanisms around a recurrent architecture: condition → state formation → persistence → reader-dependent consequence → causal return. It then reduces the unresolved conjunction to a crossed-condition RNA experiment. The two writer histories are first generated separately and then transferred into one common environment. A reader intervention tests whether the retained difference causes a downstream effect, and a second-write assay tests whether that effect returns to the chemistry of writing. Broader historical and adaptive claims are treated separately in Section 6.

Keywords: origin of biological information; RNA world; prebiotic chemistry; environmental cycling; nucleotide metabolism; ribozymes; molecular memory; causal return; molecular evolution

1. The problem: how chemistry begins to carry a history

The emergence of biological information can be viewed as part of a broader transition in which chemistry became increasingly shaped by its own prior states. Before genes, enzymes or receptors, selectivity was supplied largely by the surroundings. Experimental prebiotic systems and geochemically motivated studies show that hydration can change concentration and water activity, while mineral and metal contexts alter catalytic possibilities; pH, redox state and light can redirect which transformations remain accessible (Patel et al. 2015; Camprubi et al. 2017; Campbell et al. 2019; Morasch et al. 2019; Mrnjavac et al. 2024). When such conditions recur, reaction opportunities recur with them (Campbell et al. 2019; Attwater et al. 2025).

Candidate origin-of-life settings can also impose temporal order on chemistry (Campbell et al. 2019; Morasch et al. 2019; Attwater et al. 2025). Wet–dry cycling repeatedly concentrates reactants and can favor condensation. Thermal gradients can move RNA precursors and larger assemblies between wet and dry zones, while pH–freeze–thaw regimes can separate strands and support repeated RNA copying under supplied laboratory conditions (Campbell et al. 2019; Morasch et al. 2019; Attwater et al. 2025). Recurrence therefore exists before a molecular system can generate its own cycle.

The relevant material inventory is also chemically accessible and may reflect chemistry that is not unique to Earth. Overlapping cyanosulfidic chemistry can produce precursors of ribonucleotides, amino acids and lipids under experimentally defined conditions (Patel et al. 2015). Extraterrestrial material broadens that chemical setting: returned samples from asteroid Bennu contain all five canonical nucleobases and fourteen of the twenty protein amino acids, and later analyses identified ribose and other sugars in pristine returned material (Glavin et al. 2025; Furukawa et al. 2026). The relevance here is chemical breadth. Multiple classes of molecules later used by life belong to a wider abiotic inventory from which local environments could select.

The conceptual change begins when that chemistry makes polymers. A polymer is not merely accumulated material. Each addition changes the molecular object on which the next reaction occurs: neighboring bases alter electrostatics and protonation, sequence-dependent stacking correlates with phosphodiester reactivity, and nucleobases differ in redox susceptibility (Acharya et al. 2003; Kaukinen et al. 2003; Pitterl et al. 2010). Folding further changes which surfaces can bind or catalyze, and the populated structural ensemble can itself depend on ionic or pH conditions (Athavale et al. 2012; Snoussi et al. 2001; Monsen et al. 2022). The products of prior chemistry therefore become part of the conditions of subsequent chemistry.

From there the system can deepen without a central controller. A condition-sensitive fold creates a molecular state that is competent only when a compatible partner and substrate are present (Athavale et al. 2012; Guerrier-Takada et al. 1983). Catalysis then changes the surroundings by consuming substrates and generating products. Coupled reactions can carry the system through further states until reaction capacity is restored (Thöny et al. 2000; Wang et al. 2008; Weber et al. 2016). If one of those states persists or is copied before the cycle closes, a consequence of earlier chemistry can survive into a later one (Bostick et al. 2007; Barrangou et al. 2007; Nuñez et al. 2015).

Extant biology shows how deeply these capacities can be internalized. External ion and redox conditions become regulated pools and cofactor ratios. Condition-sensitive RNA becomes part of riboswitch and enzyme control, while transient pathway products can be converted into maintained molecular states. Dedicated readers then add specificity, and internally generated metabolic or regulatory cycles replace some of the recurrence once imposed from outside (Bird et al. 2016; Suddala & Zhang 2019; Bostick et al. 2007; Wang et al. 2008). The molecular machinery changes across these levels, but the causal progression is continuous.

The manuscript follows that causal progression rather than proposing a literal chronology. Prebiotic and reduced systems are used to establish chemical reachability; extant systems show how the same operations can be elaborated, nested and maintained. Table 1 fixes the inference boundaries for those comparisons so that they need not be repeated after every example.

The central experimental question can therefore be stated as a sequence. First, a prior condition must leave a molecular difference that survives transfer. Next, a reader must distinguish that retained state under one common environment. Finally, the reader-dependent consequence must alter a variable controlling another write. Section 4 turns those three transitions into a crossed-condition assay.

2. From response to history: the recurrent architecture

The architecture can be stated without assuming a cell. Here a write is operational shorthand for a reaction or state transition that produces the candidate molecular state later tested for persistence or discrimination; a write alone is not information, memory or a record. A response exists when a molecular state differs while a condition is present. Persistence exists when some consequence of that condition remains after the condition is removed. A condition record exists when that retained difference preserves recoverable information about which prior condition occurred under common later conditions. A reader makes the retained difference consequential when it responds differently to alternative retained states under those common later conditions. A return occurs when that consequence changes a measured variable governing a subsequent write. Recurring return creates feedback; adaptive value requires the additional demonstration that the cycle benefits a defined selectable unit. The candidate retained state need not be a unique molecular species. It can be a persistent difference in the distribution or fractional occupancy of molecular states, provided that the difference remains attributable to the prior condition after transfer. A condition record therefore creates temporal separation between cause and recoverable state: the original condition has ended, yet a molecular difference remains from which prior history can still be discriminated. Reader-dependent consequence is the next causal step.

These distinctions describe causal depth, not a proposed order of inventions. Response → path dependence → persistence → record → reader-dependent consequence → return → recurring feedback is an analytical ladder for distinguishing what a mechanism can do. Nature need not have assembled those operations in that order, and several may have coexisted or been coupled differently in different settings. Polymerization can create path dependence before a condition record exists; a stable template can carry architectural memory without recording an environmental exposure; a reader-dependent effect can occur without being copied; and selection can alter frequencies without establishing feedback. The value of the decomposition is that each demonstrated mechanism can be assigned only the capacity it actually supports.

Two additional distinctions prevent recurrence from being conflated with simple cycling. Chemical regeneration occurs when pathway flow restores a substrate, cofactor, catalyst or physical condition needed for another round of reaction. Record-dependent return is stronger: a retained state from an earlier round changes a variable governing a later write. A system can regenerate chemically without preserving which earlier condition occurred, and a molecular record can persist without regenerating the chemistry that produced it. Recurrent feedback requires both capacities to be joined across cycles.

Return also need not restore an exact molecular microstate. A cycle can regain functional write-competent capacity by regenerating a cofactor, recycling a metabolite pool or reconstructing an unmodified molecular state (Thöny et al. 2000; Wang et al. 2008; Weber et al. 2016). For the architecture defined here, functional return is sufficient if another condition-sensitive write becomes possible and the retained consequence of the previous round biases that later transition.

Figure 1 summarizes the architecture. Its arrows should be read chemically: conditions constrain accessible reactions; a write or state transition changes the molecular context; some states persist; a later interaction discriminates them; the consequence alters downstream chemistry; and coupled pathway flow can eventually change or regenerate the conditions of another write.

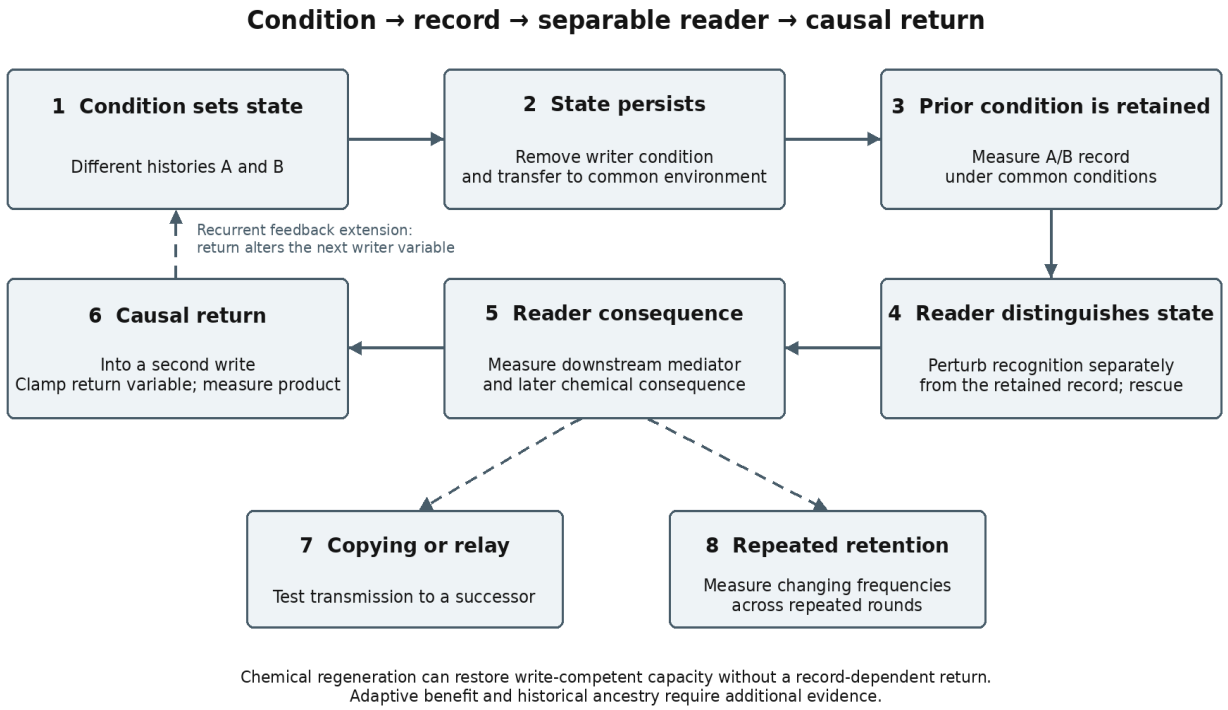

Figure 1. Operations and experimental boundaries of the proposed architecture. A condition-specific record requires state formation, persistence after removal of the producing condition, and recoverable prior-condition discrimination under common later conditions. A stronger reader-dependent consequence requires recognition that can be perturbed separately from the retained state. The second-write experiment in §4.3 tests causal return. Recurrent feedback is a further extension in which that return alters a writer variable governing a subsequent round. Copying or relay, repeated retention, adaptive benefit and historical ancestry are separate extensions. Chemical regeneration can restore write-competent capacity without constituting record-dependent return.

Table 1 sets the inference ceilings once at the outset so that the main argument can proceed without turning every mechanistic paragraph into a historical disclaimer.

Table 1. Evidence levels and inference ceilings used in this manuscript.

Claim level	Current evidence	Inference ceiling
Demonstrated chemical capacity	Activation, polymerization, copying, catalysis, compartment effects, persistent states and selective recognition are demonstrated in different reduced, synthetic or biological systems.	Shows that the relevant operation is chemically realizable in the tested context; it does not establish historical order or coupling to every other operation.
Coupled / simple-system	Selected pairs or subsets of relevant capacities have	Shows that specific capacities can coexist or causally

Claim level	Current evidence	Inference ceiling
realization	been demonstrated together in reduced systems, including environmentally cycled RNA copying, RNA–amino-acid coupling and compartment-dependent chemistry.	interact in the tested system; it does not establish the complete record–reader–return conjunction.
Prospective integrated implementation	Crossed-condition experiment with common-condition transfer, recognition perturbation/rescue and a second-write return test.	A positive result would establish the tested causal architecture in the constructed system. Geochemical provenance, autonomy, recurrence and adaptive value remain separate questions.
Historical / evolutionary inference	Comparative, phylogenetic, ancestral-reconstruction and prebiotic evidence constrain possible histories.	Historical sequence, direct ancestry and particular canonical-code assignments are not derived from mechanistic similarity alone.

Table 1 note. Representative empirical support for the demonstrated-capacity and coupled-system rows includes Campbell et al. (2019), Adamala and Szostak (2013), Attwater et al. (2025), Gianni et al. (2026), Rout et al. (2025), Singh et al. (2025), Barrangou et al. (2007) and Nuñez et al. (2015). The prospective and historical rows state inference boundaries rather than additional empirical claims.

3. Chemical capacities relevant to the recurrent architecture

Section 3 follows the progressive internalization of chemical constraint. It begins with reaction opportunities imposed by the environment and then moves to polymers whose own sequence and structure affect later chemistry. Coupled pathways introduce regeneration. Persistence carries chemical effects forward in time, reading turns those effects into later consequences, and return closes the path into another write. This is an analytical progression through causal capacities; Table 1 already defines how evidence from prebiotic, synthetic and extant systems is to be interpreted.

3.1 Environmental conditions provide the baseline chemical constraints

Before molecular products can regulate their own chemistry, selectivity lies largely outside the system. Geochemical context can open some reaction channels while suppressing others. Laboratory work summarized by Mrnjavac et al. (2024) shows that native Fe, Co and Ni can promote reactions from H2 and CO2 toward central metabolic products, while natural proton gradients across Fe(Ni)S barriers have been proposed as a mechanism for driving prebiotic carbon-fixation chemistry (Camprubi et al. 2017). Environmental chemistry can therefore sort reaction possibilities before encoded catalysts exist.

Environmental variation adds time to that selectivity. Wet–dry cycling repeatedly concentrates reactants and lowers water activity, whereas deliquescent salts can couple dehydration to spontaneous rehydration (Campbell et al. 2019). Thermal gradients around gas–water interfaces move RNA precursors and larger assemblies through concentration and drying zones (Morasch et al. 2019). In the Attwater system, pH and freeze–thaw cycling help separate strands and support repeated RNA copying (Attwater et al. 2025). The common feature is sequential access to different reaction windows.

Material availability adds a second filter. Prebiotic synthesis and extraterrestrial delivery both widen the set of compounds that can enter an abiotic inventory (Patel et al. 2015; Glavin et al. 2025; Furukawa et al. 2026). Local chemistry then determines which members of that inventory survive long enough to matter. Concentration, activation and degradation act on the pool before polymerization begins.

At this stage the system is recurrent but still externally organized. The environment determines which chemistry is accessible and cycling determines when it becomes accessible. The next transition occurs when reaction products themselves begin to constrain later reactions.

Activation chemistry provides a direct example of this selection. Bošković et al. (2026) found that different phosphate-activation routes treat canonical and noncanonical ribonucleotides differently. One route modifies nucleobases while largely sparing ribose hydroxyls; another preferentially acylates the hydroxyl groups. The same study showed condition-dependent conversion of 2-thiopyrimidines into canonical pyrimidines. The consequence is a chemically filtered substrate pool entering the next stage.

Geochemical setting reinforces that filtering. Metal composition alters reaction pathways, while pH gradients can change reaction energetics and have been proposed to facilitate specific carbon-fixation steps; concentration mechanisms and phase changes determine which products accumulate (Camprubi et al. 2017; Campbell et al. 2019; Morasch et al. 2019; Mrnjavac et al. 2024). The early substrate pool is therefore better viewed as the output of environmental selection than as a fixed starting mixture.

Repeated environmental transitions can also impose an order on those selections. A wet phase permits diffusion and exchange; drying favors condensation; rehydration redistributes the products before the next round (Campbell et al. 2019; Morasch et al. 2019). Products generated in one phase then become substrates or boundary conditions for the next. Temporal ordering can therefore precede internally generated timing.

This is the baseline from which internal regulation can emerge. Initially, the environment supplies the dominant constraints. Polymerization changes that balance because the product acquires sequence-dependent electrostatic, structural and reactive properties of its own (Acharya et al. 2003; Kaukinen et al. 2003; Athavale et al. 2012). Constraint has begun to move from the surroundings into the molecular product.

3.2 Polymerization makes the chemistry path-dependent

Polymerization is the first point at which a product begins to carry its own reaction context. Activated ribonucleotides can oligomerize in mineral-associated systems, and yields depend on variables including mineral context, pH, salinity and activation state (Burcar et al. 2015; Joshi et al. 2015). Because activation also determines which candidate nucleotides remain intact or are redirected, the polymer is assembled from an already filtered substrate pool (Bošković et al. 2026).

Each addition then changes the substrate for the next reaction. Neighboring nucleotides and phosphates shift base pKa and electrostatic context, sequence-dependent stacking alters phosphodiester reactivity, and nucleobases differ in redox susceptibility (Acharya et al. 2003; Kaukinen et al. 2003; Pitterl et al. 2010). A growing chain therefore acquires path dependence before any later process interprets it as a record.

Template-directed copying internalizes constraint further. In nonenzymatic primer extension, the template positions activated substrates beside the primer terminus, while the local chemistry determines whether extension outcompetes hydrolysis (Walton et al. 2019). Deep-sequencing methods now permit copying fidelity and accessible sequence space to be measured across experimental conditions and sequence contexts (Duzdevich et al. 2020). Under anoxic conditions, Fe^{2+} can substitute for some roles normally assigned to Mg^{2+} by supporting RNA folding, ribozyme catalysis and tested primer-extension or ligation regimes (Athavale et al. 2012; Jin et al. 2018).

The same chemical logic persists in protein polymerases. DNA and RNA polymerases use evolved active sites in which divalent-metal coordination and local substrate geometry support nucleotidyl transfer (Steitz 1998). Evolution adds fidelity control, proofreading and regulation to a reaction whose immediate success still depends on local chemical context.

The available alphabet also changes the chemistry of the polymer from the first bond onward. Ribonucleosides are not redox-equivalent: electrochemical measurements place guanosine as more readily oxidized than adenosine, cytidine or uridine under the tested conditions, and related guanine oxidation chemistry is retained in nucleotide and dinucleotide contexts (Pitterl et al. 2010). Combined with neighboring-base effects on protonation and phosphodiester reactivity, this means that a sequence is immediately a chemical context, not merely a later carrier of symbolic content.

At the extant end of this progression, nucleotide metabolism links RNA-centered chemistry directly to DNA synthesis. Ribonucleotide reductases convert ribonucleotides into deoxyribonucleotide precursors, and DNA primases synthesize the short RNA primers that initiate many replication reactions (Nordlund & Reichard 2006; Frick & Richardson 2001). An RNA-based nucleotide economy can therefore feed both the material supply and the initiation chemistry of DNA replication.

Polymerization therefore internalizes constraint in two distinct senses. A template constrains which incoming units are geometrically compatible with extension, while the growing product changes the electrostatic, structural and reactive context for later steps (Walton et al. 2019; Acharya et al. 2003; Kaukinen et al. 2003). Neither property requires the sequence to be interpreted symbolically. The polymer already has causal consequences simply because its present chemical state changes the set and rates of reactions available next. This is why path dependence precedes memory in the framework: a product can influence its own future chemistry before any later process can use it as a record of a vanished condition.

Polymer chemistry can also operate within a bounded microenvironment. Nonenzymatic RNA copying has been demonstrated inside fatty-acid vesicles when Mg^{2+} is buffered into a membrane-compatible regime, and related work shows enhanced membrane permeability to short oligonucleotide substrates under compatible conditions (Adamala &

Szostak 2013; O'Flaherty et al. 2018). Once a boundary changes local concentration, permeability or ion activity, compartment state becomes another part of the reaction context.

3.3 Folding and catalysis create compatibility windows

As polymers grow, sequence becomes structure. Nucleic acids occupy conformational ensembles rather than one fixed fold. Potassium can stabilize G-quadruplexes; protonation can favor alternative structures at acidic pH; and Fe^{2+} can support RNA folding and catalysis under anoxic conditions (Athavale et al. 2012; Monsen et al. 2022; Snoussi et al. 2001). The polymer product now participates directly in selecting which reaction is possible.

This makes coincidence central. A particular fold may be competent only when a compatible ion and reaction partner are present at the same time. The interaction therefore occupies a compatibility window in condition space. Moving outside that window can suppress the reaction or expose a different surface without changing the underlying sequence.

Catalysis lowers the amount of molecular machinery needed to exploit such windows. In-vitro evolution has produced RNA polymerase ribozymes capable of copying functional RNA (Wochner et al. 2011). QT45, a 45-nucleotide polymerase ribozyme, synthesizes its complementary strand from supplied triplets under mildly alkaline eutectic-ice conditions and can copy its own sequence at low yield when the required triplet set is provided (Gianni et al. 2026). Small RNA can therefore couple structure to chemistry in ways directly relevant to propagation.

Once structure helps select the next reaction, part of the reaction context resides in the molecular product rather than solely in the environment. That shift is the mechanistic bridge from externally imposed selectivity to internally carried chemical state.

Structure can then function as a molecular address. Noncanonical nucleic-acid folds create distinct structural surfaces; i-motif formation in cells can be pH dependent, while selective targeting of i-motif or G-quadruplex structures can alter transcriptional output in separate systems (Zeraati et al. 2018; Saha et al. 2020; Monsen et al. 2022). A change in condition can therefore alter a fold, changing molecular access without rewriting the sequence.

RNase P shows the same principle at the level of catalysis. Its RNA component can process precursor tRNA without the protein subunit when the ionic environment makes the catalytic state accessible; high Mg^{2+} can do so, as can lower Mg^{2+} supplemented by spermidine (Guerrier-Takada et al. 1983). Condition here is part of catalytic competence, not merely a background variable.

3.4 Environmental cycles can become coupled chemical cycles

Environmental cycling reopens reaction windows from outside; coupled chemistry can begin to regenerate them from within. As reactions proceed, substrates fall, products accumulate, redox state shifts and cofactors turn over (Demirel & Sandler 2002; Thöny et al. 2000; Wang et al. 2008; Xu et al. 2011). The products of one step thereby become part of the chemical setting encountered by the next.

Regeneration through a pathway need not retrace the original reaction. A sequence of forward steps can restore a depleted substrate or cofactor state. Redox metabolism repeatedly regenerates cofactor states, and tetrahydrobiopterin is restored through dedicated chemistry after oxidation (Demirel & Sandler 2002; Thöny et al. 2000). Regeneration is therefore a trajectory through intermediate states rather than microscopic reversal.

This creates a second source of chemical recurrence. Environmental forcing can reopen a reaction window, but a coupled network can also rebuild the conditions needed for another round. Metal-driven nonenzymatic analogues of central metabolic chemistry show that pathway-like transformations are available even before a modern enzyme complement is introduced (Muchowska et al. 2017).

The integration is therefore a flow, not a wiring diagram. A reaction changes the state through which later reactions must pass. If one of those states persists long enough to be copied or selectively read, pathway history can become molecular history.

Energetic coupling alone does not yet create history. Free-energy input can sustain a nonequilibrium trajectory (Demirel & Sandler 2002), but some material difference must survive that trajectory to be encountered later. The carrier may be a sequence or covalent modification; it may instead be a conformational population or compartment state. What matters is persistence beyond the condition that produced it.

Forward regeneration is visible in specific metabolite cycles. Methyl transfer consumes S-adenosylmethionine and produces S-adenosylhomocysteine; a riboswitch can discriminate the inhibitory product and regulate genes involved in its recycling (Wang et al. 2008). Fe(II)/2-oxoglutarate-dependent oxidation similarly consumes 2-oxoglutarate and generates succinate, while 2-hydroxyglutarate can compete with 2-oxoglutarate and inhibit members of the same enzyme family (Xu et al. 2011). The products and side-products of one step can therefore become conditions for later steps rather than disappearing from the causal description.

Restoring a writable state can itself require several forward reactions. TET enzymes oxidize 5-methylcytosine through successive intermediates; TDG then excises an oxidized base and base-excision repair restores unmodified cytosine (He et al. 2011; Weber et al. 2016). The example makes the logic explicit: functional regeneration can be achieved through a new path rather than reversal of the original write.

3.5 A nucleotide-derived economy links pathway state to heredity

Nucleotide chemistry is positioned to couple these transitions because the same molecular family participates in heredity and in the chemistry that powers or redirects reactions. Here, 'nucleotide-derived economy' refers to that recurring participation rather than to a single interchangeable pool. Ribonucleotides become RNA; ribonucleotide reductases convert ribonucleotide precursors into DNA precursors (Nordlund & Reichard 2006); many coenzymes contain nucleotide-derived moieties, a feature long discussed in hypotheses about earlier nucleic-acid-centered metabolism (White 1976); and NAD+, NADH and desphospho-CoA can enter RNA directly as noncanonical 5′ caps (Bird et al. 2016).

That overlap allows metabolite identity to be written directly onto a covalent RNA feature. NAD+, NADH and desphospho-CoA can initiate transcription as noncanonical 5′ caps, and the NAD cap can later be removed by DXO-mediated deNADding, promoting transcript decay (Bird et al. 2016; Jiao et al. 2017). A metabolite identity has thereby become a molecular feature that later chemistry can distinguish.

The same economy reaches the amino-acid layer. Aminoacyl-tRNA synthetases use ATP-dependent adenylation to activate amino acids before transfer to RNA adaptors, and indirect pathways can modify attached precursors on the tRNA itself (Sheppard et al. 2008). Ribonucleotide reductase simultaneously diverts part of the ribonucleotide supply toward DNA, linking the precursor economy to another hereditary polymer. Diphthamide-dependent translation of the RNR large subunit RRM1 further links deoxyribonucleotide production to translational state (Zhao et al. 2024).

Across increasing degrees of organization, the locus of control therefore shifts inward. Environmental chemistry first constrains reaction space; polymers and folds add molecular context; coupled pathways regenerate reaction capacity; and extant regulation stabilizes these relationships with dedicated sensors, enzymes and maintenance systems (Campbell et al. 2019; Walton et al. 2019; Athavale et al. 2012; Wang et al. 2008; Bostick et al. 2007). Table 2 summarizes that progression.

Table 2. Chemical capacities across increasing degrees of molecular organization.

Context	Relevant constraint	Capacity illustrated	Illustrative mechanism
Environmental chemistry	Hydration, temperature, light, pH, redox, metals, substrate concentration	Condition-dependent reaction selection	Wet–dry / freeze–thaw cycling; mineral and activation chemistry
Polymer-centered chemistry	External conditions plus sequence and fold	Template context, structural compatibility, catalytic surfaces	Template copying; condition-sensitive RNA folds; ribozymes
Coupled chemical networks	External forcing plus products, cofactors and compartments	Metabolite ratios, activated intermediates, local pH/redox, substrate regeneration	Nucleotide-derived cofactors; coacervates; pathway cycling
Extant biological regulation	Internally regulated chemical state plus environmental inputs	Dedicated sensors, adaptors, readers, maintenance and feedback	tRNA/T-box control; chromatin and RNA marks; signaling and repair

Table 2 note. Representative sources by row: environmental chemistry (Campbell et al. 2019; Attwater et al. 2025); polymer-centered chemistry (Walton et al. 2019; Athavale et al. 2012; Gianni et al. 2026); coupled chemical networks (Wang et al. 2008; Wang et al. 2026); extant regulation (Suddala & Zhang 2019; Bostick et al. 2007; Chen et al. 2020).

Modern cells provide direct examples of metabolic state controlling molecular writing. Acetyl-CoA availability changes histone acetylation through ATP-citrate lyase-dependent carbon flow (Wellen et al. 2009). Oxygen availability constrains KDM6A activity, while 2-hydroxyglutarate competes with 2-oxoglutarate-dependent dioxygenases and

shifts DNA or histone methylation states (Chakraborty et al. 2019; Xu et al. 2011). Metabolic state can therefore regulate both the installation and removal of persistent molecular features.

Metabolic and hereditary chemistry also share material pathways. Histidine biosynthesis begins with ATP and phosphoribosyl pyrophosphate (PRPP), while PRPP is also a precursor for de novo purine synthesis; the two pathways are additionally connected by AICAR, which is produced in both but used in purine biosynthesis (Vázquez-Salazar et al. 2018). The nucleotide–amino-acid boundary is therefore chemically porous before the translation mapping itself is considered.

The architectural value of this economy lies in repeated physical coupling. ATP-dependent amino-acid activation links nucleotide chemistry to bond formation (Sheppard et al. 2008). Many coenzymes contain nucleotide-derived moieties (White 1976), while NAD+, NADH and desphospho-CoA can be incorporated directly as noncanonical RNA caps (Bird et al. 2016). Ribonucleotide reduction then routes part of the same precursor economy toward DNA (Nordlund & Reichard 2006). Transient chemistry is repeatedly connected to molecules that can persist, be copied or be selectively recognized.

Because the same chemical families participate in several roles, coupling does not require wholly separate systems for heredity, metabolism and adaptor chemistry to meet for the first time. New regulatory relationships can be built onto molecules and reactions already participating in material and energetic flow.

3.6 RNA–amino-acid coupling creates a multifunctional coordination interface

RNA and amino-acid chemistry can be coupled without the full modern translation apparatus. Direct affinities between selected RNA motifs and some amino acids provide one route to selective association (Yarus et al. 2009). Engineered aminoacylating ribozymes provide another experimental demonstration by attaching amino acids to RNA (Ohuchi et al. 2007). More recent work shows reciprocal chemistry in the opposite direction: amino acids can accelerate polymerization of plausible ribonucleotide substrates, and thioester chemistry can generate aminoacyl- and peptidyl-RNA in water (Rout et al. 2025; Singh et al. 2025).

Once an adaptor system exists, tRNA turns that cross-domain chemistry into a reusable interface. Aminoacylation couples ATP-dependent activation to a defined RNA structure. T-box riboswitches read both tRNA identity and charging state, while tRNA modifications can alter codon-biased translation and stress-response outputs (Suddala & Zhang 2019; Begley et al. 2007; Thompson & Gottesman 2014). The same molecular class can even be recruited to prime reverse transcription (Abbink & Berkhout 2008).

The interface can also reach the compartment. In several bacteria, aminoacyl-phosphatidylglycerol synthases transfer amino acids from aminoacyl-tRNA to membrane phosphatidylglycerol, changing membrane charge and stress resistance (Roy 2009). An adaptor state can therefore be routed into a physical property of the boundary that shapes later chemistry.

tRNA is useful here because evolution repeatedly recruits the same adaptor class at different interfaces. It participates in sensing and amino-acid transfer, supports decoding, and in some systems reaches membrane chemistry or DNA synthesis (Suddala & Zhang 2019; Giegé et al. 1998; Roy 2009; Abbink & Berkhout 2008). An RNA-centered coupling can thus become a control node as organization increases.

The adaptor layer also shows that catalytic access and identity discrimination can be separated experimentally. The idea of an operational RNA code locates amino-acid identity in acceptor-stem and related tRNA features rather than requiring the anticodon alone (Schimmel et al. 1993). In the Nanoarchaeum equitans alanyl system, an RNA minihelix containing the acceptor branch supports strong G3:U70-dependent charging by the assembled enzyme, whereas a shorter substrate retains weaker pair-independent activity with one subunit (Arutaki et al. 2020). The shorter substrate demonstrates access to the chemistry; the larger minihelix demonstrates how a reader can impose identity on that chemistry. Specificity can therefore be added to an existing capacity rather than appearing as an indivisible property.

Adaptor and reader can remain functionally matched despite substantial structural change. Mammalian mitochondrial serine tRNAs depart strongly from the canonical cloverleaf, including loss of the D arm in one isoacceptor and loss of the elongated variable arm normally used by seryl-tRNA synthetases. The cognate mitochondrial enzyme uses a correspondingly altered recognition strategy (Chimnaronk et al. 2005). This extant pairing shows that noncanonical adaptor structures can be accommodated by a reader architecture that uses different recognition elements, demonstrating flexibility in how recognition capacity can be physically implemented.

Modern tRNA identity makes the same point at finer resolution. Recognition determinants are distributed across the acceptor stem, discriminator base, anticodon and family-specific positions rather than residing in a single universal key (Giegé et al. 1998). An adaptor can therefore acquire or lose one determinant while retaining others, and a cognate reader can alter the weight it assigns to those features. This distributed architecture matters for origin scenarios because specificity need not appear fully formed. Catalytic access, coarse molecular discrimination and high-fidelity identity can be layered progressively onto the same RNA–amino-acid interface.

Charging state then adds a second variable on top of identity. A T-box does not merely recognize which tRNA is present; it also distinguishes whether the acceptor end is aminoacylated (Suddala & Zhang 2019). The same RNA object can therefore carry both a relatively stable identity and a rapidly changing metabolic state. That combination is particularly relevant to the framework: one molecular scaffold can preserve what it is while simultaneously reporting what has recently happened to it, allowing downstream chemistry to respond to state without erasing identity.

3.7 Persistence turns a chemical trajectory into molecular history

A chemical trajectory becomes history when one of its consequences survives the event that produced it. Simple material persistence means that a molecule remains present. Template persistence is stronger because an older sequence constrains a new copy. A condition record adds another requirement: after alternative writer conditions have ended, the retained state must still distinguish which history occurred. All three cases begin with the same physical fact—a consequence outlives its cause.

Metabolite-linked states show a second route. Noncanonical RNA caps provide a covalent molecular feature whose identity can alter transcript processing or turnover: NAD+, NADH and desphospho-CoA can cap transcripts during initiation, and NAD-capped RNA can be recognized and deNADded by DXO (Bird et al. 2016; Jiao et al. 2017). Other covalent RNA modifications can likewise alter later recruitment. At DNA breaks, TRDMT1-dependent m5C supports recruitment of RAD51 and RAD52, and RAD52 shows enhanced binding to m5C-containing DNA–RNA hybrids in the cited system (Chen et al. 2020).

CRISPR joins several of these operations in one extant system. Foreign-DNA-derived spacer sequence is integrated into CRISPR arrays, persists in the locus, and spacer content later determines sequence-specific phage resistance (Barrangou et al. 2007; Nuñez et al. 2015). Spacer acquisition, persistence and a sequence-dependent downstream consequence are therefore jointly realizable in one extant system.

Template memory is not a separate mechanism added after copying; it is intrinsic to templated synthesis. An existing strand constrains the sequence of the new strand, and copying fidelity determines how much of that organization survives repeated rounds. In RNA-evolution experiments, earlier polymerase variants lacked sufficient copying fidelity to propagate functional information, whereas an improved-fidelity variant maintained heritable information through repeated replication and selection (Papastavrou et al. 2024). Persistence therefore has a quantitative dimension: a carrier may survive physically while its specific sequence information is progressively lost.

Extant maintenance methylation makes the reconstruction logic unusually explicit. After replication, a previously methylated CpG is hemimethylated because the parental strand retains the mark and the daughter strand does not. UHRF1 recognizes that asymmetric state and recruits DNMT1 to restore methylation on the new strand (Bostick et al. 2007; Sharif et al. 2007; Avvakumov et al. 2008). The state persists not because the original mark is immortal, but because an older state is read to reconstruct a successor state. That distinction between material persistence and informational persistence is central to the manuscript's broader definition of history.

Persistence can also be distributed across a molecular assembly. Replication-coupled histone recycling transfers parental histones and associated states to daughter chromatin, and perturbing that machinery alters silencing, histone-pattern inheritance and differentiation outcomes (Charlton et al. 2024; Wen et al. 2023). Molecular history can therefore survive through controlled partition and reconstruction rather than through DNA sequence alone.

Persistence is governed by kinetics and maintenance, not simply by covalent stability. Turnover or dilution can erase a durable mark, whereas active copying or repair can preserve a chemically short-lived state. Condition-associated history can also be statistical: defined environmental agents can generate characteristic mutational-signature distributions (Kucab et al. 2019). A record can therefore reside in a shifted population of molecular states rather than in one unique product.

3.8 Reading makes retained history consequential

A persistent state becomes consequential when later chemistry responds differently to it. No symbolic interpretation is required. The operation is physical: one state presents a binding or catalytic opportunity that another state does not.

RNA provides a compact example. A T-box combines sequence recognition of a cognate tRNA with sensing of the acceptor-end charging state and changes transcription or translation accordingly (Suddala & Zhang 2019). Modified RNA can also alter Toll-like-receptor activation in a nucleoside-state-dependent manner (Karikó et al. 2005). In both cases, chemical state changes which later interaction is accessible.

Extant systems implement this discrimination in several ways. Chromatin-associated proteins recognize molecular marks, CRISPR spacer content can determine sequence-specific antiviral resistance, and tRNA identity elements constrain charging and decoding (Bostick et al. 2007; Barrangou et al. 2007; Suddala & Zhang 2019; Giegé et al. 1998). Modified RNA at DNA breaks can likewise redirect repair-associated interactions (Chen et al. 2020). Dedicated readers add specificity to the same underlying operation: state-dependent molecular recognition.

That operation gives a direct experimental criterion. Remove the writer conditions and place both histories into one common read environment. If the retained difference is causal, disrupting the proposed recognition step should eliminate the downstream contrast without destroying generic chemistry, and restoring recognition should restore the contrast.

The earliest reader need not be a dedicated protein. Template pairing, structure-dependent recognition and metal-dependent catalysis can all generate selective molecular responses without a dedicated protein reader (Walton et al. 2019; Athavale et al. 2012; Saha et al. 2020). For the stronger reader claim tested in Section 4, however, recognition must be experimentally separable from the state being recognized. That intervention distinguishes a readable record from chemistry in which persistence and response are inseparable.

The common-condition requirement is what separates that operation from continued environmental responsiveness. If the original writer condition remains present, a downstream difference may simply reflect ongoing pH, ion or substrate effects. Transfer removes that ambiguity. Once both histories face the same later environment, any reproducible difference must reside in the material states that survived the transfer. Perturbing the reader then asks whether that retained difference is merely detectable or is actually causal for what happens next.

Reader specificity can depend on a structural feature that is absent from a monomeric precursor. Poly(ADP-ribose) provides a clear example: the RNF146 WWE domain recognizes the internal iso-ADP-ribose linkage of PAR rather than monomeric ADP-ribose, and this binding allosterically activates the ligase (Wang et al. 2012; DaRosa et al. 2015). The molecular state therefore becomes consequential when it contains the feature the reader recognizes.

Written states can redirect access to repair machinery. At DNA breaks, TRDMT1-dependent RNA methylation supports recruitment of RAD51 and RAD52, and RAD52 shows enhanced binding to m5C-containing DNA–RNA hybrids relative to unmodified hybrids in the cited system (Chen et al. 2020). G-quadruplex structures are likewise implicated in DNA-damage and repair pathways (Monsen et al. 2022). These cases show how a molecular state can bias which downstream machinery gains access.

Architectural memory can even change carriers. In Oxytricha, somatic-genome development involves extensive DNA rearrangement (Chen et al. 2014). Maternal DNA and RNA templates influence that reconstruction, and experimentally supplied alternative templates can reprogram rearrangement outcomes (Nowacki et al. 2008). Prior organization can therefore be relayed through a different molecular carrier and used to guide later reconstruction.

3.9 Return paths close the architecture

Return closes the architecture by feeding a reader-dependent consequence back into the chemistry of a later write. The altered variable may be a resource state, catalytic capacity or local physicochemical condition. If the relation recurs, it becomes feedback; if the feedback also improves persistence or reproduction of a defined unit, it becomes adaptive.

This definition separates return from ordinary metabolite-dependent writing. The key test is whether a retained state changes a downstream process that, in turn, modifies a later writer variable. Extant regulation supplies components of such paths: regulator abundance and cofactor state can reshape later molecular writing (Wellen et al. 2009; Chakraborty et al. 2019; Xu et al. 2011), while the SAH riboswitch provides a product-sensing regulatory edge back toward

coenzyme recycling (Wang et al. 2008). Section 3.5 established the forward edge from metabolic state to write; return asks for the causal path back.

The SAH riboswitch provides a concrete return edge. S-adenosylhomocysteine, produced by S-adenosylmethionine-dependent methyl transfer, is sensed by a riboswitch that activates genes involved in coenzyme recycling (Wang et al. 2008). A chemical product is read into a regulatory consequence that changes machinery acting on the same metabolic state. What remains to be added for the full architecture is a retained prior-condition record upstream of that edge.

Return can proceed through forward chemistry. Biochemical systems restore write-competent capacity through additional reactions rather than microscopic reversal (Thöny et al. 2000; Wang et al. 2008; Weber et al. 2016). Pathway flow and hereditary-state chemistry can therefore become coupled when a retained-state consequence alters the pathway that supplies or regenerates a later write.

Return therefore changes the status of the retained state. Before return, the state can influence a downstream event but remains terminal with respect to the writing process. After return, the consequence reaches back into the chemistry that determines a later state-forming event. That connection is the first point at which molecular history can modify the conditions under which new history is produced. Recurrence adds another requirement: the altered writer variable must persist or be regenerated strongly enough for the relation to operate again in a subsequent cycle.

Taken together, Section 3 has moved from externally imposed reaction windows through polymer context, coupled chemistry, persistent states, readers and return. The progression is analytical rather than historical, but it reveals a common direction in causal organization: chemistry becomes increasingly conditioned by products of its own prior states. Table 3 consolidates the demonstrated capacities and marks where that evidence stops.

Table 3. Demonstrated capacities, later elaborations, and the remaining architectural gap. Here return means a downstream consequence alters a later writer variable; recurrent feedback additionally requires that edge to operate across another cycle.

Relevant capacity	Reduced / prebiotic-relevant demonstration	Extant elaboration or integration	Remaining gap
Material supply	Overlapping nucleotide/amino-acid/lipid precursor chemistry; asteroidal nucleobases, amino acids and ribose	Biosynthetic and salvage networks	Concentration and integration in a specific origin setting
Environmental cycling	Wet–dry, thermal and pH–freeze–thaw regimes alter reaction accessibility	Metabolic, cell-cycle and signaling oscillations	Transition from imposed to self-maintained cycles
Polymerization / copying	Activated RNA growth; template-directed extension; ribozyme replication	Protein polymerases with proofreading and repair	Autonomous substrate supply and sustained replication
Catalysis / gating	Condition-sensitive RNA folding; short ribozymes	Riboswitches, RNPs, enzymes and receptors	Origin of catalysts and coupling among reaction windows
RNA–amino-acid coupling	RNA affinity, ribozyme/thioester aminoacylation, peptidyl-RNA	tRNA/aaRS, T-box control, membrane lipid aminoacylation	Historical route to stable adaptor mappings
Compartment coupling	Fatty-acid vesicles; coacervate microenvironments	Membranes, organelles and regulated transport	Autonomous growth, division and coupling to records
Persistence / reading	Copying and stable products; partial differential retention	Maintenance marks, CRISPR, RNA/chromatin readers	Condition-specific record plus reader in one simple system
Return / recurrent feedback	Pathway-like nonenzymatic reaction sequences are demonstrated in reduced chemistry; record-linked causal return remains prospective.	Extensive metabolic and regulatory feedback	Causal return in one simple system; recurrent closure across a subsequent cycle.

Table 3 note. Representative support by capacity: material supply (Patel et al. 2015; Glavin et al. 2025; Furukawa et al. 2026); environmental cycling (Campbell et al. 2019; Morasch et al. 2019; Attwater et al. 2025); polymerization/copying (Burcar et al. 2015; Walton et al. 2019; Gianni et al. 2026); catalysis/gating (Athavale et al. 2012; Wochner et al. 2011); RNA–amino-acid coupling (Yarus et al. 2009; Ohuchi et al. 2007; Rout et al. 2025; Singh et al. 2025); compartment coupling (Adamala & Szostak 2013; O'Flaherty et al. 2018; Wang et al. 2026); persistence/reading (Bostick et al. 2007; Barrangou et al. 2007; Nuñez et al. 2015; Chen et al. 2020); reduced pathway-like chemistry and extant return/regeneration examples (Muchowska et al. 2017; Wang et al. 2008; Weber et al. 2016).

Repeated cycles add differential retention. In supplied RNA-replication systems, copying fidelity changes whether functional sequences persist across generations (Papastavrou et al. 2024). Cyclic phase separation can also enrich particular sequences through repeated partitioning even without autonomous replication (Bartolucci et al. 2023). Once variants exist, recurrence can therefore change which states remain represented.

Recent experiments also bring formerly separate chemical domains into closer contact. Amino acids accelerate polymerization of prebiotically plausible ribonucleoside 2′,3′-cyclic phosphates (Rout et al. 2025), while thioester chemistry can capture activated amino-acid chemistry directly on RNA as aminoacyl- and peptidyl-RNA (Singh et al. 2025). RNA formation and amino-acid chemistry can therefore influence one another without a modern translation system.

Environmental recurrence and RNA catalysis can likewise operate in the same experiment. Attwater et al. (2025) used coupled pH and freeze–thaw cycles with a polymerase ribozyme to support repeated RNA copying. Gianni et al. (2026) then showed that a 45-nucleotide polymerase ribozyme can synthesize its complementary strand and, with a defined triplet substrate set, a low-yield copy of its own sequence. These systems join environmental forcing, strand management and catalytic copying within progressively more integrated regimes.

Compartment state supplies another internally generated condition. Charged polymers within peptide coacervates can create uneven H^+/OH^- distributions, producing local pH differences that can be used to control reaction cascades inside the condensed phase (Wang et al. 2026). A molecular assembly can therefore alter the reaction environment experienced by its own contents.

These integrations substantially narrow the gap. Several adjacent steps are already coupled in reduced systems, including environmentally cycled RNA copying, RNA–amino-acid coupling and compartment-dependent chemistry (Rout et al. 2025; Singh et al. 2025; Attwater et al. 2025; Gianni et al. 2026; Wang et al. 2026). What has not been demonstrated in one controlled system is the complete transition from retained history to reader-dependent consequence and then back into a later write. Section 4 therefore asks whether that missing conjunction can be reconstructed directly.

4. The decisive experiment: reconstructing the architecture

The experiment preserves the causal order of the framework while separating its tests. Two writer histories, A and B, are generated independently; transfer into one common environment asks whether their difference persists; a reader intervention asks whether the retained state is causally recognized; and a second-write assay asks whether the reader-dependent consequence returns to the chemistry of writing.

The decision boundaries remain deliberately separate. A molecular state that persists after transfer but does not preserve recoverable prior-condition information is persistence alone. Recoverable A/B information under a common read condition satisfies the condition-record criterion. Reader-dependent consequence requires a separable recognition step whose disruption removes the state-dependent effect and whose rescue restores it. A measured change in a later writer variable establishes return; recurrence and adaptive benefit require additional tests.

A successful implementation would establish the record–reader–return architecture in nonliving chemistry. The programme then proceeds in stages. Phase 1 uses engineered components where necessary to test chemical realizability. Phase 2 replaces those conveniences one at a time with simpler or geochemically supported components while keeping the causal criteria fixed. Historical provenance and adaptive value are evaluated only after that mechanistic chain has been established.

4.1 Competing models and simpler explanations

The experiment distinguishes three outcomes before the molecular implementation is introduced. An equilibrium response produces an A/B difference only while the writer condition remains. An analog (graded) condition record retains a quantitative A/B difference after both histories are transferred into the same environment but has no separable reader-dependent consequence. The record-plus-reader model predicts both a retained prior-condition difference and a causal recognition-dependent consequence under the common read condition. Operationally, the experiment therefore asks two questions in sequence: does a writer-history difference persist after transfer, and does a separable reader make that retained difference consequential? Sections 4.2 and 4.3 then introduce the RNA-ligation implementation, define its molecular components and quantitative variables, and map those measurements onto the causal criteria. Discrimination of chemically different record kinds would be a stronger extension, but it is not what this first implementation claims.

Each simpler explanation has a matched control. State reconstruction tests whether the candidate retained state is sufficient by rebuilding the relevant state levels from independently characterized components and placing them into processed backgrounds from both writer arms; equivalent outputs across backgrounds are required once the candidate state and total material are matched. Selective erasure tests whether the proposed state is necessary. Recognition disruption and compensatory rescue isolate the reader from generic catalytic competence. Time-resolved measurements and functional carryover controls test whether residual writer chemistry, degradation, renewed writing or substrate imbalance can explain the downstream contrast. The exact molecular forms of these controls are specified after the ligation system is introduced in Sections 4.2 and 4.3. Repetition alone does not establish feedback, and enrichment alone does not establish adaptation.

A compartment can be added without changing these definitions. Coacervates and fatty-acid vesicles provide distinct physical environments in which concentration, permeability, ionic conditions and reaction kinetics can differ from the bulk environment (Adamala & Szostak 2013; O'Flaherty et al. 2018; Wang et al. 2026). Compartmentalization counts as evidence for memory only when a retained historical distinction is identified and causally read after the producing condition has ended.

The systems reviewed above occupy different positions along this sequence. Some establish persistence, some add reader-dependent consequences, and others combine reading with copying or recurrence. Supplementary Tables S1–S2 classify those branches explicitly. None of the simple systems reviewed there reaches the full condition-record → separable reader → return chain, which is why Section 4 targets that conjunction directly.

4.2 Crossed-condition record and reader test

The critical test asks whether one selected chemical implementation can produce a retained record with a separable reader-dependent consequence. The primary writer is a pH-gated RNA ligation using the same carrier C, short donor D, activation chemistry and Mg2+ concentration in both arms; covalent joining of C and D produces ligated product L. The writer history is therefore represented by the fraction of starting carrier converted to L, quantified as q = L/C0, where C0 is the independently quantified starting carrier amount for that batch; Gate 1 asks whether the A/B difference in q survives transfer into a common environment. Pilot characterization begins near pH 8.5 and pH 7.0 and is used only to identify a reproducible, non-saturating contrast. The confirmatory pair is then frozen before unblinding. If that preregistered implementation fails the materiality criterion, a separately preregistered Mg2+-contrast implementation serves as the fallback.

Prepare otherwise matched RNA reactions under writer environments A and B, then quench and transfer both products into one common read environment. Validate removal of free writer reagents and residual activity, and verify chemical equilibration of pH, ionic composition and activation chemistry. Transfer validation includes carrier-free A/B process blanks that receive identical clean RNA and reader after processing, spike-ins of plausible residual writer species at their measured analytical upper bounds, and known C/D/L mixtures used to quantify species-specific recovery. A no-pre-existing-L control is carried through the read and second-write interval to measure any renewed C → L formation under second-stage conditions. After transfer, A-derived and B-derived samples must experience the same future reaction conditions except for effects caused by the retained molecular state itself. Equalize total carrier and downstream substrate opportunities while preserving the candidate record-state fraction. The retained A/B distinction must remain above the prespecified Gate 1 materiality threshold for a common-condition persistence interval fixed during the pilot and frozen before confirmatory unblinding. Both populations then meet the same reader. Selective state erasure should remove the consequence, whereas reconstruction of the matched state from characterized C/D/L should reproduce it. Recognition disruption should abolish the state-dependent difference without destroying generic catalysis, and a compensatory recognition rescue should restore the reader-dependent consequence without restoring, rewriting or changing the abundance of the original record state.

A clean condition → retained state → reader-dependent consequence from nonliving reagents would establish the record-and-reader portion of Phase 1. Section 4.3 then tests whether that reader-dependent consequence can alter a second write, while §4.4 treats recurrence, adaptive benefit and greater prebiotic plausibility.

Interpretation follows the staged assay. A contrast explained by carryover, unequal carrier abundance, degradation, renewed writing or nonspecific catalytic failure is non-diagnostic. Reader support requires validated transfer plus selective recognition perturbation and rescue; causal return additionally requires a measured effect on the second write. Recurrence is reserved for a later experiment in which that consequence alters the writer variable of a subsequent round.

Confirmatory design is batch-level and preregistered. Pilot batches estimate variance, dynamic range and dose-response shape but are not reused for confirmatory testing; the materiality margins, sample size, exclusions, transformations, stopping rules and primary contrasts are then frozen before unblinding. Exact statistical definitions are given in Methods.

4.3 Candidate ligation implementation and causal return

A first implementation uses the pH-gated RNA ligation introduced in §4.2 as the writer; here ligation refers only to the covalent joining of C and D. The primary state variable remains total-input-normalized ligation, q = L/C0. The intact-state ligation fraction L/(Cintact + L), where Cintact denotes unligated intact carrier C, is retained as a secondary diagnostic. Carrier-derived degradation products and total recovery are measured by mass balance, so differential degradation cannot create an apparent positive Gate 1 result simply by shrinking the denominator. Before confirmatory data are unblinded, a minimum acceptable interval for total carrier recovery is frozen as a batch-level QC criterion from the pilot; batches outside that interval fail QC rather than contributing to the record contrast. After quench and transfer, L, intact C, donor, degradation products and residual activation chemistry are quantified independently at transfer and again at the prespecified persistence endpoint. Gate 1 is passed only if the A/B contrast remains above its prespecified materiality margin δq after the common-condition interval while both arms satisfy the frozen recovery and equilibration criteria.

In the reader-return stage, retained L engages reader gate G, G releases helper H, and H promotes ligation of fresh substrates X and Y into second-write product P2. The reader must operationally distinguish the ligated product from matched noncovalent alternatives. A laboratory-first implementation can do this with a junction-spanning strand-displacement gate. Gate G sequesters helper oligonucleotide H. Ligated product L places a short trigger domain and a branch-migration domain in cis across the C–D junction, creating a geometry that can release H from G. Unligated C and D are tested as free, co-bound and splinted controls at the same concentrations and binding opportunities, and those controls are followed through the complete reader and second-write interval to verify that they remain unligated.

Toehold exchange and allosterically gated strand displacement provide established ways to tune this kind of enzyme-free nucleic-acid release reaction (Zhang & Winfree 2009; Yang et al. 2016). The reader gate G can be DNA or a DNA/RNA hybrid and is treated explicitly as a laboratory convenience, not as a prebiotic claim. Reader validation uses characterized ligation products in the complete reciprocal comparison of original versus junction-mutant trigger with original versus compensatory gate. For each gate, basal leakage, helper loading and releasable-helper capacity are measured before writer-history experiments. Pure L and the matched fragment controls determine whether the system truly distinguishes the ligated geometry rather than merely responding to sequence abundance or proximity. The chemical identity of L, including junction linkage or regioisomer composition where the ligation chemistry permits alternatives, is characterized rather than inferred from product length alone. Gate 2 asks whether the retained A/B state difference produces a reader-dependent consequence: the A/B effect must be present with competent recognition, lie within the prespecified equivalence bound when recognition is disrupted, and be restored by the compensatory gate. Exact contrasts and margins are defined in Methods.

The released helper gives the readout a direct chemical role in the next write. H is a short splint complementary to fresh substrates X and Y, positioning their ligatable ends and increasing their effective molarity under the same activation chemistry used for the second ligation. Thus the common read reaction is L + G(H) → L:G + Hfree, followed by H-assisted X + Y → P2. Whether H turns over catalytically or remains partly product-bound is measured rather than assumed. Newly formed P2 is distinguished from retained first-write material. Equalizing Hfree across the A and B arms should remove the second-write contrast while leaving initial-record recognition intact; the clamp is set within the pilot-defined responsive region and validated over the full observation interval rather than at one endpoint, with a sham-clamp control for effects of the intervention itself. Supplying H independently should restore P2 when the reader-to-helper connection is interrupted, using a helper exposure matched to the validated responsive range rather than an arbitrarily saturating dose. In helper-free reactions, L, L:G and gate fragments are tested for direct effects on X + Y ligation. Direct measurement of Hfree makes the mediator test quantitative; any Hfree reporter is first shown not to materially sequester helper or alter the reaction.

Feasibility before confirmation. The circuit only works if three things coexist: the A/B writer difference must survive transfer, the reader must release enough helper above background leakage, and the helper must move the second ligation through a responsive part of its dose-response curve. Each quantity is measurable in the staged pilot. The

operating-range model used to combine them is given in Methods so that the main text can stay focused on the causal logic; confirmatory inference comes from the preregistered gate contrasts rather than from model fit.

The pilot measures the writer contrast, ligated-versus-fragment reader discrimination, basal gate leakage, helper loss and the helper dose-response of the second ligation. Those measured ranges are then used to ask whether any parameter regime satisfies all three experimental gates. The operating-range model predicts a helper dose-response that may saturate or, under splint excess, peak and decline; slower helper release after a junction mismatch; a lag between helper release and second-product formation; and collapse of the A/B difference when helper is clamped to the same level in both arms. If no measured regime satisfies the prespecified margins, this reader-helper implementation is rejected before a long confirmatory campaign.

Gate 3 tests whether the A/B difference in newly formed second-write product depends on the helper pathway. The comparison is made with the reader-helper connection intact and with helper clamped to the same level in both arms; the sign, materiality margin and observation time are fixed from the pilot. L must predict free helper, free helper must predict newly formed P2 over the prespecified time course, and independent helper rescue must restore P2 when release from G is blocked. Passing these tests establishes a causal write → read → write return connection. Phase 1 stops at that edge. Recurrent closure is a separate extension because the pH writer used here has no validated P2 → pH return chemistry; any recurrence implementation must show that P2 changes a defined writer variable strongly and persistently enough to alter the next write, and that clamping that writer variable abolishes the dependence without disrupting the preceding record–reader–return chain. A candidate Mg2+ implementation is considered with the downstream extensions in §4.4.

4.4 Illustrative recurrence, adaptive and prebiotic extensions

Recurrent closure requires one additional causal edge beyond Gate 3. Free Mg2+ is one candidate writer variable, but RNA binding of Mg2+ is not sufficient by itself. P2 production would have to shift free Mg2+ activity beyond the buffering and sequestration imposed by the remaining RNA, phosphate, activation reagents and salts, and the shift would have to persist long enough to move the next writer reaction across a responsive region of its measured Mg2+ dose-response. Independent manipulation of P2 should change both free Mg2+ and the next write in the predicted direction, whereas experimentally clamping free Mg2+ should abolish that dependence without disrupting the preceding record–reader–return chain.

The adaptive extension asks whether the one-step write → read → write connection benefits a selectable compartment. Fatty-acid vesicles provide one defined selectable unit and net membrane growth over a challenge cycle is the primary benefit endpoint. RNA-loaded fatty-acid vesicles can acquire membrane from relaxed competitors through osmotic effects (Chen et al. 2004), providing an experimentally grounded growth coupling. Before the record circuit is interpreted adaptively, however, P2 must be shown independently to change a prespecified growth-driving variable in this implementation, such as retained solute content, osmotic state, membrane acquisition or another quantified coupling; if manipulating P2 at otherwise matched composition does not change that variable, vesicle growth cannot be attributed to the record → P2 path. Initial vesicle size, membrane composition, RNA loading and osmotic state are matched, and membrane acquisition is distinguished from swelling, fusion and selective loss of smaller vesicles. The record circuit is then compared with a recognition-disabled control, a constitutively active helper control and an informative-versus-scrambled or misleading record comparison matched for chemical composition and resource expenditure under challenge sequences matched in chemistry and frequency but differing in temporal correlation. Carrier and helper exchange are measured so any growth advantage remains associated with the vesicle lineage that retained the record. Reproduction or multigeneration selection is a later endpoint rather than a prerequisite for the record-reader claim.

A reaction ledger constrains the Phase 2 prebiotic extension. Mechanistic success is reported separately from prebiotic plausibility, and engineered components are replaced one at a time while the Gates 1–3 causal criteria remain fixed so that loss of function identifies which convenience currently carries the mechanism. Each requirement is classified as demonstrated in a specified geochemical setting, plausibly supplied but not demonstrated there, or a laboratory convenience. For every transition the ledger records pH, temperature, metal and salt concentrations, activation chemistry, reaction time, concentration method, transfer losses and catalyst source. Surface photochemistry, mineral reactions, vesicle copying and coacervate chemistry are combined only when a compatible sequence or an explicit transport/concentration step is demonstrated. Supplied purified catalysts remain declared laboratory operations until simpler replacements preserve the same record, read and return behavior. Those experimental boundaries also clarify

how the framework should be compared with theories that address the later emergence and stabilization of the genetic code.

5. Relation to existing theories of the code's origin

Once molecular distinctions can persist and influence later chemistry, a later problem becomes possible: how particular RNA triplets came to map onto particular amino acids. Stereochemical models address the accessibility of some assignments through direct RNA–amino-acid affinity (Yarus et al. 2009). Coevolution models instead follow biosynthetic relationships (Wong 1975), whereas error-minimization models ask how selection acts among competing mappings (Freeland & Hurst 1998; Freeland et al. 2000). Other models address fixation, reassignment and expansion once a coding system exists or begins to become established. Frozen-accident and reassignment models address constraints after mappings become embedded (Crick 1968; Osawa & Jukes 1989; Schultz & Yarus 1996), while Higgs (2009) reconstructs stepwise routes by which selection during code expansion could yield an error-minimizing code.

These mechanisms can act at different stages of the same later transition. Affinity can bias which pairings are locally accessible (Yarus et al. 2009). Biosynthetic structure can restrict reachable assignments (Wong 1975), while selection can favor mappings with lower mistranslation or mutation costs (Freeland & Hurst 1998; Freeland et al. 2000). Once a mapping is distributed through a larger system, communal spread and historical contingency can constrain subsequent change without making codon reassignment impossible (Crick 1968; Osawa & Jukes 1989; Vetsigian et al. 2006).

The framework developed here sits one causal level earlier. Before any codon assignment can be stabilized by affinity, selection or contingency, molecular distinctions must persist long enough to be recognized and to alter later chemistry. The record–reader–return experiment tests that prerequisite rather than the identity of any particular codon assignment.

Table 4. Scope map: related theories, questions, and relevant observations.

Model or framework	Primary question / contribution	Outside its scope by itself	Relevant observation or test
Stereochemical	RNA–amino-acid binding sites show enrichment of some cognate codon/anticodon triplets, providing a stereochemical route that could bias a subset of assignments.	Why affinity is incomplete across the full table; historical fixation; condition-record memory.	Direct affinity should predict assignment or enrichment for the residues it explains, independent of later selection.
Coevolution	Biosynthetic precursor-product relations can constrain which amino acids and assignments become reachable together or sequentially.	Why one particular persistent molecular record should be read later; assignment fixation outside metabolic route structure.	Assignments should track biosynthetic family history beyond what stereochemistry/error costs predict.
Error minimization / selection	Selection can favor code structures that reduce the phenotypic cost of mutation and mistranslation after variant mappings exist.	How the first persistent mapping/reader relation is generated; why particular assignments become reachable before selection.	Alternative mappings with lower error cost should be selectively enriched once mapping variants are available.
Frozen accident / historical contingency	Near universality can arise because early mappings become costly to change after translation depends on them, although codon reassignment can still occur.	Why the original mapping has the chemical/statistical structure it has; a memory architecture before fixation.	Near universality together with rare lineage-specific reassignments is consistent with strong but incomplete historical constraint.
Condition–record–reader–return architecture (this paper)	Specifies an experimental decomposition of condition-specific records, recognition and causal return in candidate chemistry.	Does not derive codon assignments or independently originate the causal-information criterion.	A/B retention, selective recognition and consequence under common conditions; a separate intervention tests causal return into a second write.
Semantic information (Kolchinsky and Wolpert 2018)	Environmental correlations causally supporting system maintenance.	Does not specify this candidate RNA chemistry.	Scrambling correlations changes the maintenance endpoint.
Causal leverage (Bartlett 2024, preprint)	Information interventions change future trajectories without requiring a viability endpoint.	Does not establish the proposed prebiotic implementation.	Erasure or scrambling changes the distribution of subsequent trajectories.

Table 4 note. Representative sources: stereochemical models (Yarus et al. 2009); coevolution (Wong 1975); error minimization (Freeland & Hurst 1998; Freeland et al. 2000); frozen accident and reassignment (Crick 1968; Osawa & Jukes 1989; Schultz & Yarus 1996); stepwise code-expansion model (Higgs 2009); semantic information (Kolchinsky & Wolpert 2018); causal leverage (Bartlett 2024, preprint).

These theories can therefore be arranged by the stage of the mapping problem they constrain. Affinity affects local accessibility. Biosynthetic structure narrows the reachable space, and selection acts once competing mappings have consequences (Wong 1975; Freeland & Hurst 1998; Freeland et al. 2000; Yarus et al. 2009). Communal innovation sharing provides one proposed route to code universality and refinement (Vetsigian et al. 2006), while frozen-accident logic and the deleterious effects of reassignment can constrain later change once a code is embedded in translation (Koonin & Novozhilov 2009). The present architecture supplies the earlier condition that makes such a history possible at all.

Metabolic chemistry has also motivated hypotheses about the transition into coding. White (1976) proposed an earlier metabolic system composed of nucleic-acid enzymes and interpreted nucleotide-containing coenzymes as possible vestiges of it; Szathmáry (1993) proposed amino-acid-charged RNA adapters acting as coenzymes of ribozymes before translation. These proposals illustrate possible routes by which the RNA–metabolite–amino-acid couplings described above could be incorporated into a later coding system; they are origin hypotheses rather than demonstrations of that historical route. In the present framework, the experimentally accessible question is whether a condition-associated molecular state can persist, be read under common later conditions and alter another write.

Intervention-based accounts of information provide the closest formal comparison. Kolchinsky and Wolpert (2018) ask whether environmentally correlated information causally supports system maintenance, while Bartlett's causal-leverage formulation asks whether interventions on an information-bearing variable change later trajectories. The present manuscript gives those causal relations a concrete molecular decomposition: writer condition, retained state, reader and return.

Chemical memory supplies another neighboring concept. Hysteresis and bistability can allow a system's present state to depend on its past in a chemically fueled crystallization system (Schnitter et al. 2022), while synthetic strand-displacement circuits can preserve temporal information in engineered nucleic-acid populations (Lapteva et al. 2022). The crossed-condition experiment adds causal separation: the producing condition is removed, both histories enter one later environment, and the reader is perturbed independently of the retained state. That design assigns the path from past condition to present state to future consequence.

Autocatalytic-set theory asks whether a reaction subsystem is both autocatalytic and sustainable from an ambient food set (Hordijk & Steel 2004), while cooperative-replicator experiments show self-assembling RNA replicators forming cooperative catalytic cycles and networks (Vaidya et al. 2012). A history-bearing network adds another capability: its future reaction regime can depend on a retained consequence of an earlier condition. Joining self-maintenance to that retained-state control is therefore a later step toward biological adaptation.

6. Boundaries and open questions

The preceding sections separate mechanism from chronology by construction. The evidence establishes chemical capacities at defined levels of organization, and the crossed-condition experiment asks whether three critical capacities can be joined in one system (Adamala & Szostak 2013; Attwater et al. 2025; Gianni et al. 2026; Rout et al. 2025; Singh et al. 2025). Historical continuity among particular implementations remains a separate question for comparative, phylogenetic, ancestral-reconstruction and geochemically constrained evidence.

The principal experimental gap is correspondingly specific. Among the simple systems reviewed here, none joins a retained prior-condition record to a separable reader-dependent consequence and then returns that consequence into a later write. Section 4 targets exactly that conjunction. Autonomous catalyst production, geochemical provenance and the individual assignments of the canonical genetic code lie outside that test.

A positive Phase 1 result would establish the minimal mechanistic claim: nonliving molecular chemistry can convert a prior environmental difference into a persistent state, read that state after transfer and use the consequence to alter a later write. Phase 2 then asks whether the same causal chain survives replacement of engineered conveniences by simpler or geochemically supported components. Adaptive extensions ask a different question again: whether carrying the state improves persistence or reproduction.

Failure is also interpretable by edge. The writer may fail to generate sufficient state separation; the state may decay during transfer; the reader may fail to discriminate it; or the readout may be too weak to alter the next write. A failed circuit therefore rejects that implementation at a defined edge. Broader falsification requires multiple mechanistically distinct implementations with independently validated components and prespecified operating ranges.

Adaptive significance begins only after the minimal record–reader relation is established. A retained molecular distinction can be causal without benefiting the system that carries it. Kolchinsky and Wolpert's semantic-information framework asks whether system–environment correlations causally support a specified viability or maintenance criterion under information-scrambling interventions (Kolchinsky & Wolpert 2018). In the present experimental extension, that stronger level is operationalized with prespecified persistence or growth endpoints for a defined vesicle population.

7. Conclusion

The starting point is externally organized chemistry. Environmental cycles determine when reaction windows open, while local chemistry filters which substrates remain available (Patel et al. 2015; Campbell et al. 2019; Morasch et al. 2019; Attwater et al. 2025). Polymerization then moves part of that constraint into the product itself because neighboring sequence changes electrostatic and protonation context and phosphodiester reactivity, while nucleobase identity changes redox susceptibility (Acharya et al. 2003; Kaukinen et al. 2003; Pitterl et al. 2010). From that point onward, the products of chemistry increasingly help set the conditions for what can happen next.

Coupled pathways deepen that transition by restoring reaction capacity through forward chemistry. Cofactors can be regenerated, products can become substrates, and molecular states can be rebuilt through multistep pathways (Thöny et al. 2000; Wang et al. 2008; Weber et al. 2016). If a state persists or is copied before the cycle closes, the pathway acquires a material link to its own past.

Nucleotide-derived chemistry is especially effective at carrying that link across domains. Nucleotides form hereditary polymers and participate in energy transfer and amino-acid activation; many coenzymes contain nucleotide-derived moieties, metabolite nucleotides can enter RNA as noncanonical caps, and ribonucleotide reduction connects ribonucleotide precursors to DNA (White 1976; Sheppard et al. 2008; Bird et al. 2016; Nordlund & Reichard 2006). Later tRNA-dependent systems show how an RNA-centered interface can coordinate metabolic state with decoding, stress-responsive translation and membrane chemistry (Suddala & Zhang 2019; Begley et al. 2007; Roy 2009).

The mechanisms reviewed in Section 3 can therefore be read as stages in one recurrent architecture. External conditions shape a write; the resulting molecular state changes later reaction possibilities; persistence carries part of that state forward; and a reader converts the retained difference into a consequence. When that consequence changes the chemistry of another write, the system has acquired return. The outstanding experiment is to place those edges in one controlled chain.

The origin-of-information problem can therefore be placed earlier than the origin of a completed code. The first transition of interest is from chemistry that responds only to its present conditions to chemistry whose future depends on a retained consequence of its past. The crossed-condition experiment asks whether that transition can be constructed in one molecular system. A positive result would establish history-bearing chemistry as an experimentally realizable causal architecture.

8. Methods

Prospective record-reader-return assay (§4). The exact confirmatory quantities are retained here rather than in the conceptual narrative. Gate 1 uses total-input-normalized ligation $q = L/C0$, where C0 is the independently quantified starting carrier amount, and tests the signed batch-level difference $\Delta q = \text{mean}(qA) - \text{mean}(qB)$ against a prespecified materiality margin δq. L/(Cintact + L), where Cintact denotes unligated intact carrier C, is reported as a secondary intact-state ligation fraction together with total carrier recovery and carrier-derived degradation products. The pilot defines the final A/B pH pair, a common-condition persistence interval, acceptable post-transfer equilibration criteria for pH/ionic/activation variables, and a minimum acceptable total-carrier recovery interval; all are frozen before confirmatory unblinding. Gate 1 is evaluated at the end of that persistence interval, not only immediately after quench. Batches that fail the recovery or equilibration criteria fail prespecified QC and are not interpreted as evidence for or against the record. Let Y denote the preregistered primary reader outcome, with its units and observation time frozen before confirmatory unblinding. Gate 2 uses the reader interaction contrast $\Delta R = [(YA - YB)\text{competent} - (YA - YB)\text{recognition-disrupted}]$ with a prespecified margin δR. Gate 2 additionally requires the competent-arm A/B simple effect to exceed its own pilot-frozen materiality margin in the expected direction, the recognition-disrupted A/B contrast to lie within pilot-frozen equivalence bounds, and compensatory rescue to restore the A/B effect while the rescued-versus-competent difference lies within a frozen equivalence bound. Gate 3 uses the return contrast $\Delta RET =$

[(P2A − P2B)intact − (P2A − P2B)H-clamped] with a prespecified margin δRET, where intact denotes the unmanipulated reader-helper connection. Gate 3 additionally requires the reader-helper-intact A/B simple effect to exceed its own pilot-frozen materiality margin in the expected direction, the H-clamped A/B contrast to lie within pilot-frozen equivalence bounds, and independent helper rescue to restore the second-write effect within a frozen quantitative rescue interval. Positive materiality criteria require the lower bound of the signed 95% confidence interval to exceed the relevant margin; abolition/equivalence criteria use two one-sided 5% tests, equivalently requiring the 90% confidence interval to lie wholly inside the prespecified equivalence bounds. A pilot estimates batch variance, correlation and dose-response shape; confirmatory sample size is then set by batch-level simulation and frozen before unblinding. Because the claim requires all three gates to pass, the confirmatory decision is an intersection-union test; testing each component at level α preserves a level-α test for the conjunction, so no additional multiplicity correction is required for declaring that conjunction (Berger & Hsu 1996), although all gate-specific estimates and intervals are reported. A/B processing order and reader assignment are randomized within preparation blocks, sample identity is blinded during measurement and quantification, and all conditions are distributed across experimental days and reagent lots. The pilot-frozen protocol, margins, QC criteria, operating ranges and analysis code will be timestamped in an OSF preregistration before confirmatory unblinding.

Mechanistic control implementation (§§4.1–4.3). Reconstruction uses independently characterized C, D and L to rebuild pilot-defined high- and low-q mixtures; each mixture is tested in processed A- and B-derived backgrounds after matching q, total carrier and available donor. Writer-origin dependence at matched q is treated as evidence for an additional retained variable rather than support for ligation fraction alone. Transfer controls include carrier-free A/B process blanks followed by identical clean RNA and reader, spike-ins at the analytical upper bounds of plausible residual writer species, and known C/D/L mixtures for species-specific recovery. A no-pre-existing-L control is sampled across the full read/second-write interval to quantify reactivation of first-write ligation. Selective-erasure experiments include a sham treatment and verify that erasure reagents or products do not independently suppress reading. Reader validation uses the full original/mutant trigger × original/compensatory gate matrix and measures basal leakage, helper loading and releasable-helper capacity for each gate. Fragment controls are assayed through the complete interval to confirm that they remain unligated. Helper clamping is validated by time-resolved free-helper measurements within the prespecified responsive range, includes a sham clamp, and is accompanied by helper-free tests for direct effects of L, L:G or gate fragments on X + Y ligation.

Reader-helper kinetics (§4.3). The helper is a splint, so excess helper can inhibit productive ligation by partitioning X and Y into separate binary complexes. The operating-range model therefore does not assume a monotone Michaelis response. A minimal mass-action description uses $H + X \rightleftharpoons HX$, $H + Y \rightleftharpoons HY$, $HX + Y \rightleftharpoons HXY$ and $HY + X \rightleftharpoons HXY$, with $d[P2]/dt = k0[X][Y] + kcat[HXY]$ and mass conservation for H, X and Y, where k0 denotes the background ligation rate constant and kcat the ternary-complex-assisted rate constant. This scheme permits a productive ternary complex to peak and then fall as total H increases. Pilot data are used to determine whether the observed helper dose-response is monotone, saturating or bell-shaped and to identify one monotonic operating limb away from any turnover point. The confirmatory helper range, expected contrast sign and stopping rules are then frozen before unblinding; a circuit is not scored as failed merely because an unmodeled excess-splint regime reverses the sign.

9. Declarations

Data and code availability. No new empirical or computational data were generated for this manuscript. The argument relies on the cited primary literature and on prospective assay definitions specified in §8. Earlier computational analyses from development versions are retained separately at https://doi.org/10.5281/zenodo.20966659 for transparency; they are superseded development materials and are not evidentiary support for the claims retained in this version.

Funding. This research received no external funding.

Competing interests. The author declares no competing interests.

Author contributions. D.L. directed the research programme, developed and curated the framework with the assistance described below, selected questions and analyses, integrated the manuscript, and is responsible for its content. Computational implementation and drafting assistance are identified in the AI-assistance disclosure.

AI-assistance disclosure. Large language models assisted this work under the author’s direction. Anthropic’s Claude supported literature retrieval, analysis-code development during earlier manuscript development, synthesis and

drafting. OpenAI's ChatGPT supported critical review, literature checks, revision of the arguments and prospective experimental designs, and preparation and checking of this document, including figures. Model-generated suggestions informed some hypotheses and interpretations. AI assistance is not independent validation; cited experiments and prospective proposals retain their distinct evidentiary status. The author is responsible for reviewing and approving the manuscript and its supporting materials.

Classification. Biology; Evolutionary Biology; Molecular Biology.

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Supplementary Table S1. Experiment-level evidence trace supporting Supplementary Table S2

Representative rows identify the tested system, retained variable, intervention or comparison, observed outcome, and the evidentiary boundary used in the main table. Supplementary Table S2 retains the broader branch classification.

Example / system	History type	Retained variable	Intervention / comparison	Observed outcome	Source and boundary
Condition-sensitive nucleic-acid fold / G4–i-motif	state response	Folded nucleic-acid structure	Change K+, pH or structure-stabilizing condition; compare structural/functional	Condition-sensitive structure formation; in separate systems, stabilization of such	Monsen et al. 2022; Zeraati et al. 2018; Saha et al. 2020. These sources establish

Example / system	History type	Retained variable	Intervention / comparison	Observed outcome	Source and boundary
			state	folds alters transcriptional output	related structural and functional capacities in different systems; persistence after washout and a separable reader are not established.
NAD-capped RNA	covalent state	5′ NAD cap on transcript	Compare capped/uncapped initiation and decapping/turnover machinery	Covalent cap changes transcript fate and is processed by dedicated enzymes	Bird et al. 2016; Jiao et al. 2017. Prior-condition discrimination after common-condition transfer is untested.
Maintenance methylation	architectural memory	Hemimethylated CpG / parental-strand mark	Perturb UHRF1/DNMT1 recognition and maintenance machinery	Parental-strand state is recognized and copied to daughter strand	Sharif et al. 2007; Bostick et al. 2007; Avvakumov et al. 2008. External-exposure record is outside scope.
Exposure mutation signature	condition-associated sequence	Substitution spectrum retained in DNA	Expose matched cells to defined agents and sequence descendants	Many agents produce characteristic mutation distributions	Kucab et al. 2019. The signature records exposure but is not itself shown to be a causal reader.
CRISPR spacer acquisition	environmental record	Invader-derived spacer in CRISPR array	Acquire or alter spacer content; compare resistance to sequence-matched phage	Phage-derived spacers are acquired, and addition or removal of matched spacer sequence changes phage resistance	Nuñez et al. 2015; Barrangou et al. 2007. Repeated selection series is not established by the cited experiments.
Oxytricha reconstruction	architectural memory	Maternal DNA/RNA templates and rearranged somatic organization	Manipulate maternal templates during macronuclear development	Maternal templates can reprogram descendant somatic-genome reconstruction	Nowacki et al. 2008 (template manipulation); Chen et al. 2014 (somatic-genome architecture). External-condition record is outside scope.
Cyclic phase separation	differential retention	Sequence-dependent partitioning through repeated cycles	Repeat phase separation and quantify enrichment	Selected sequences enrich through repeated physical partitioning	Bartolucci et al. 2023. Lineage-level adaptive benefit and a separable reader are untested.
RNA-templated DNA repair	template transfer	Transcript sequence used at DNA break	Alter donor transcript abundance or repair factors	RNA oligonucleotides or transcripts can template DNA repair in yeast and mammalian/human systems	Meers et al. 2020; Chakraborty et al. 2023; Jalan et al. 2025. Prior-condition record is untested.
RNA modification / innate sensing	covalent state	Modified nucleotide state in RNA	Compare modified and unmodified RNA in receptor assays	Modification state alters innate-receptor activation	Karikó et al. 2005. No general self/foreign rule or environmental record is established.

Supplementary Table S2. Evidence branches reached by selected cited examples. Status codes: D, demonstrated in the cited system; 0, explicitly tested with no support at the stated sensitivity; U, untested or not established by the cited experiment; O, outside the scope of that history type.

Example	History type	Record	Reader effect	Copy/relay	Repeated retention	Recurrent feedback	Benefit / scope
Condition-sensitive nucleic-acid fold / G4–i-motif	state response	U	U	O	O	U	Persistence after condition removal and a separable reader remain untested.
Metabolite or NAD cap	covalent state	U	D	U	U	U	Covalent state and reader-mediated fate are demonstrated; crossed-condition record is untested.
Maintenance methylation	template / architectural memory	O	D	D	U	U	Parental-strand state is read and copied; external-exposure record is outside scope.
Exposure mutation signature	condition-associated sequence	D	U	D	U	U	Prior exposure can be inferred from sequence distributions; causal reading by the signature itself is untested.
CRISPR spacer acquisition	environmental record	D	D	D	U	U	Foreign-DNA-derived spacer is integrated into the CRISPR array, and spacer content later determines sequence-specific resistance; repeated selection series is untested.
Oxytricha RNA-guided reconstruction	architectural memory	O	D	D	U	U	Maternal carriers guide reconstruction; external-condition record is outside scope.
Cyclic phase separation	differential retention	U	U	U	D	U	Repeated enrichment is demonstrated; lineage-level benefit is untested.
RNA-templated repair	template transfer	U	D	D	U	U	RNA transfers sequence information into DNA repair; condition-specific history is untested.
RNA modification / innate sensing	covalent state	U	D	U	U	U	Modification-dependent recognition is demonstrated; environmental memory is untested.